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Transplant rejection in HLA-identical recipients

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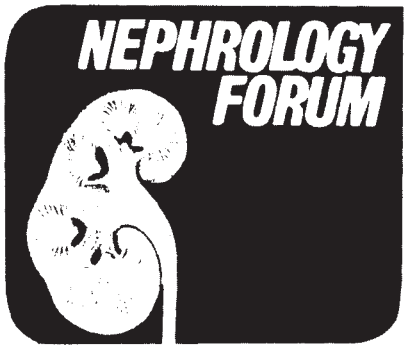

Case presentation

Case 1. A 32-yr-old woman was admitted to New England Medical Center Hospital (NEMCH) for renal transplantation from her 27-yr-old brother after 5 months of dialysis treatment.

Approximately 8 months earlier, renal biopsy revealed an advanced proliferative crescentic sclerosing glomerulonephritis. Immunofluorescent studies revealed segmental, often peripheral, granular deposition of immunoglobulins (especially IgA and IgM) and complement (C3). Prednisone therapy was begun, but the patient developed progressive renal failure. Prednisone was discontinued, and chronic hemodialysis was initiated 3 months after the biopsy.

The following tissue-typing data were available prior to transplantation:

	ABO blood group	Lymphocyte antigens
Recipient	A	All, Bw35, Cw4/A10 (Aw25), Bw16
Donor	A	All, Bw35, Cw4/A10 (Aw25), Bw16

Pretransplant "cross-match" test was negative.

Ten days following transplantation, the serum creatinine concentration was 1.3 mg/100 ml, and the patient was discharged on a regimen of prednisone (60 mg/day) and azathioprine (100 mg/day).

One day following discharge, the patient noted blood-tinged urine, mild graft site tenderness, and decreased urine output. Two days later, fever developed and the patient was readmitted to the hospital. The renal graft site was no longer tender, and there was no obvious swelling. Results of urinalysis revealed 20 white blood cells/high power field and two to three coarse granular casts. The serum creatinine concentration was 3.1 mg/100 ml. Serum concentrations of calcium, phosphorus, uric acid, transaminases, and total protein and albumin were normal. An i.v. urogram showed a very faint nephrogram of the transplanted kidney at 18 min with no evidence of obstruction. Methylprednisolone was administered in an i.v. dosage of 1 g daily, for 3 days; oral administration of prednisone and azathioprine was continued. One day following admission, a renal scan revealed adequate perfusion with poor

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function. Renal scans repeated 1 and 2 weeks later revealed neither perfusion nor function, despite administration of a second i.v. course of methlprednisolone therapy (1 g daily, for three days). Chronic hemodialysis therapy was reinstituted, and oral administration of prednisone and azathioprine was discontinued.

Five weeks after transplantation, transplant nephrectomy was performed. Histologic sections were consistent with diffuse interstitial rejection and vascular rejection. There was subtotal infarction with focal areas of both arterial and venous thrombosis. Two days after transplant nephrectomy (3 weeks after discontinuing administration of immunosuppressive agents), a mixed lymphocyte culture reaction between the patient and the kidney donor revealed a stimulation index of 2.5 and a relative response of 6%—a "negative" test result.

Case 2. A 20-yr-old woman was admitted to NEMCH for renal transplantation from her 19-yr-old brother.

Approximately 2.5 years earlier, renal biopsy had revealed light and immunofluorescent microscopic findings consistent with membranoproliferative glomerulonephritis. Electron microscopy had disclosed subepithelial and intramembranous electron-dense deposits with focal areas of basement-membrane-splitting.

The following tissue-typing data were available prior to transplantation:

	ABO blood group	Lymphocyte antigens
Recipient	A	A1, A29, B17, B40
Donor	O	A1, A29, B17, B40

Pretransplant "cross-match" test was negative: a mixed lymphocyte culture reaction was "negative" with a stimulation index of 1.98 and a relative response of 29%.

Within 48 hr after transplantation, the serum creatinine level fell to 1.0 mg/100 ml. An i.v. urogram and renal scan showed excellent transplant function with no evidence of vascular or urinary tract obstruction. Two weeks later, the serum creatinine concentration was 0.8 mg/100 ml, and the patient was discharged on a regimen of prednisone (50 mg/day), azathioprine (150 mg/day), propranolol, and hydralazine.

Two days following discharge, the patient noted fever, chills, and diffuse myalgia, and she was readmitted. The initial serum creatinine concentration was 1.0 mg/100 ml, but rose rapidly to 5.0 mg/100 ml. Methylprednisolone was administered in an i.v. dosage of 1 g daily, for 4 days, but the serum creatinine concentration increased progressively, and the patient became oliguric. Hemodialysis was initiated; oral administration of azathioprine and prednisone was continued. Multiple cultures of blood and urine were sterile. Renal scans showed adequate perfusion with poor function. Two weeks after admission, a second i.v. course of methylprednisolone therapy was administered (1 g daily, for 2 days). During the next 3 days, the serum creatinine concentration stabilized and then fell without further dialysis. Four weeks following admission, the patient was discharged. One month later, the serum concentration of creatinine was 0.9 mg/100 ml.

Discussion

Introduction. Because of the high rate of success when HLA-identical siblings are selected as donors for kidney transplantation, relatively little attention has been paid to the fact that not all such transplantations are successful. These two cases illustrate that serious rejection episodes can occur in well-matched patients, leading in some cases to loss of a graft. The overall incidence of graft failure from rejection in HLA-identical sibling transplant patients is around 15%, and two thirds of these represent early failures (10% overall) within the first 6 weeks after transplantation [1]. Those patients who experience transplant rejection do not differ significantly in clinical manifestations or in the pathologic appearance of the rejecting kidney, when compared to individuals grossly mismatched for the HLA antigens.

Of course, one must be certain that other causes of renal failure in the graft have been considered. *First*, the usual work-up to rule out infection or obstruction must be performed. One very interesting form of infection that may mimic rejection is related to cytomegalovirus (CMV). In this syndrome, there may be decreased renal function with proteinuria, elevated hepatic enzymes, lymphocytosis, granulocytopenia, and fever. Documentation of a rise in antibody titer to CMV is essential to confirm the diagnosis in retrospect. *Second*, one should consider whether recurrent or *de novo* glomerulonephritis is affecting the transplant. This is an interesting topic in itself, but it is not our purpose to review it here, today. In the present cases, there is no evidence that glomerulonephritis was responsible for decline in graft function.

Our main concern today revolves around the question, Why are HLA-identical sibling grafts rejected? Before answering this question, it is neces-

sary to review the current status of the human major histocompatibility complex (MHC).

Major histocompatibility complex. On the short arm of chromosome 6, there is a region known as HLA containing the genes for the strong transplantation antigens in man (Fig. 1). The main evidence for the strength of these antigens comes, in fact, from the success rate of transplantation between HLA-identical siblings compared with the lower success rate when antigens of this region are mismatched [1]. The two primary classes of antigens are defined by their function and biochemical structure. The old nomenclature identified Class I antigens as SD, serologically defined antigens. Class II antigens were identified as LD, lymphocyte-defined antigens. Because Class II antigens are now serologically definable, a new nomenclature has become necessary.

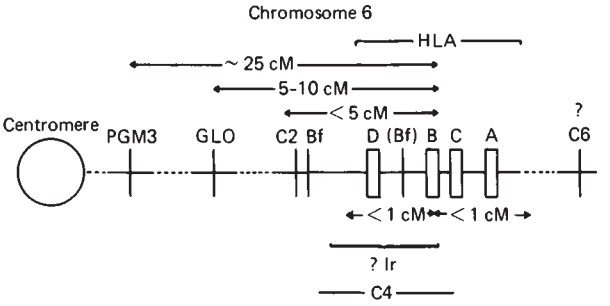

Fig. 1. *Short arm of the human chromosome 6.* The *HLA* region contains genes for three Class I loci (A,B,C) and one Class II locus (*D*), which is defined by the mixed lymphocyte culture reaction (MLR). Class II serologically defined antigens exist on B lymphocytes and monocytes, which are D-related (DR). Some of the enzyme and complement polymorphisms are also shown. cM = centimorgan map unit.

Class I antigens are the classical serologically defined HLA antigens of the A, B, and C loci, and they are expressed on the surfaces of virtually all body cells. The molecular structure for these antigens consists of a light-chain (β_2-microglobulin; mol wt, 12,000 daltons) and a heavy-chain (mol wt, 33,000 daltons) that contains the antigenic specificity. Growing evidence suggests that Class I antigens function in normal immunobiology to focus the effector mechanism in dealing with cells that have been infected with viruses and possibly cells that have undergone neoplastic transformation as well.

Class II antigens promote proliferation *in vitro* in the so-called mixed lymphocyte culture reaction (MLR) (Fig. 2). Class II antigens are related to the D locus and have a molecular structure consisting of two polypeptide chains of similar size (mol wt, 23,000 and 30,000 daltons). Antigens of the D locus

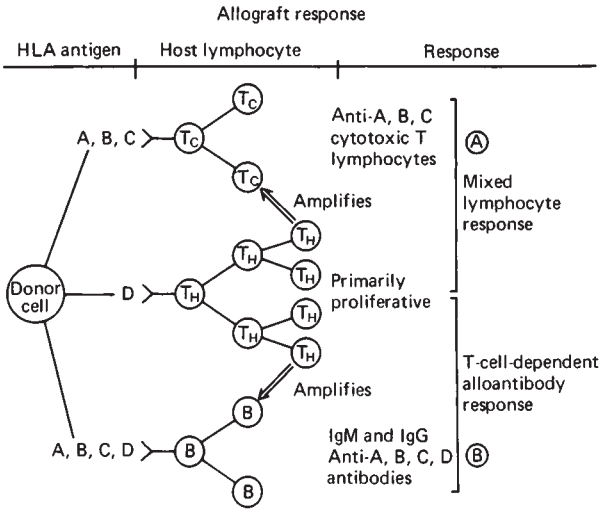

Fig. 2. *Schema of the relative roles of HLA-A,B,C,D antigens in initiation of the alloimmune response and in the development of effector cells and antibodies.* Two main classes of T lymphocytes recognize antigens: T_C, which are the precursors to the cytotoxic "killer" cells; and T_H, which are the helper cells for amplication of the cytotoxic response. T_H also provide help to *B* lymphocytes for production of a fully mature *IgG* response. Note that T_C generally recognize Class I antigens, while the T_H signal is provided principally by the *D* antigens, which have Class II antigens closely associated with them.

in man are analogous to antigens of the I region in the mouse. In the I region of the mouse, genes controlling immune responses to certain antigens have been demonstrated (Ir genes) [2]. The cell surface structures, which are alloantigens, are called Ia. It is now clear that the surface Ia structures, which are limited in distribution to B lymphocytes and monocytes, being absent from T lymphocytes and platelets, play an important role in cell-to-cell interactions during the induction of the immune response.

Additional genes of immunologic interest in the HLA region are the certain complement of the components: C2, Bf of the alternative pathway, C4, and possibly C6. The C3b receptor for activated C3 is also coded for by genes somewhere in the HLA region (Fig. 1).

There are over 50 defined antigenic specificities for the Class I-A, B, and C loci and over 10 specificities for the Class II-D locus [3]. These antigens are inherited by simple Mendelian codominance, and the cluster of antigens obtained by genetic transmission from each parent is called a *haplotype* (Fig. 3). Each individual has two antigens of the A locus, two of the B locus, two of the C locus, and two of the D locus. The number of permutations and combinations of these antigens is extremely high, and

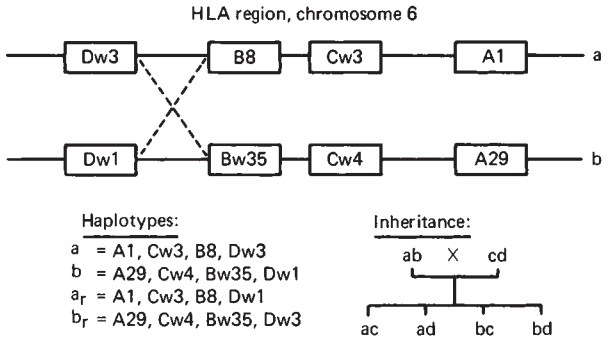

Fig. 3. *Inheritance of HLA haplotypes.* Each chromosomal segment of linked genes is termed *a haplotype*, and each individual inherits one haplotype from each parent. The *A,B,C,D* antigens of haplotypes *a* and *b* are shown for a hypothetical individual in chromosomal order on the diagram, and also shown below as they would be written in text. If individual *ab* were to marry *cd*, their offspring would be four types only, as far as HLA is concerned. Occasionally, recombination occurs in the germ line of a parent, resulting in an altered haplotype (*recombination represented by broken lines*). The frequency of recombinant children is a measure of the map distance (1% recombination frequency = 1 cM).

one can see how difficult it is to obtain a complete match for all eight antigens between unrelated individuals. In families, however, 25% of siblings are identical for the entire HLA region; in other words, they have the same haplotypes from their parents. By the same token, 25% are totally dissimilar for HLA, and the other 50% are semi-identical, sharing one haplotype and differing for the other haplotype. In approximately 1% of offspring, disruption of the alleles inherited with a given haplotype occurs because of recombination during meiosis in one of the parents; in other words, an offspring receives portions of both parental haplotypes as a result of crossing over. In transplantation matching for example, when two siblings are identical for HLA-A,B,C but differ for HLA-D, they cannot be considered HLA identical because they are mismatched for that portion of the MHC that is crossed over. The frequency of recombination in normal families is an indication of the relative distance, or map distance, in the chromosomal segment, and, as stated earlier, HLA-A,B,C,D recombination occurs in approximately 1% of offspring.

In a study of the relative roles of the Class I and Class II antigens in human skin-grafting, Van Rood has demonstrated that when skin grafts are exchanged between unrelated people who have been carefully typed for the antigens of the HLA region, there is an important effect of the HLA-D locus [4]. For example, if individuals have the same Class I antigens of the A,B,C loci but are mismatched for the Class II antigens of the D locus, the survival of the graft seems to be directly related to the degree of responsiveness in the MLR. The reverse situation, a Class II match and a Class I mismatch, results in some prolongation of graft survival. In contrast, after immunization of the Class I antigens of the A,B,C loci assume major importance because immunity to Class I antigens shortens graft survival, whereas immunity to the Class II antigens of the D locus is of less importance.

It is necessary to remember that there is one other major histocompatibility system, the ABO blood group system, which is important because the A and B substances have been shown to be present on the endothelium of blood vessels. In the present discussion of antigen matching, we are assuming there is compatibility for the ABO system as well because this genetic system is routinely matched in tissue-typing laboratories.

Rejection in HLA-identical sibling transplant patients. The early rejection rate of HLA-identical kidney transplants, as stated earlier, seems to be around 10% [1]. Brief review of four recently published series may help to put this in perspective. In a study of 26 HLA-identical sibling transplant patients, Cheigh et al reported six graft failures [5]. Two of the grafts underwent accelerated acute rejection; one was described pathologically as a Shwartzman reaction, and the other was associated with a staphylococcal sepsis. A third graft failed through acute rejection within the first week of transplantation, similar to our first case today. The other three grafts were chronically rejected, failing with typical obliterative endarteritis. Mixed lymphocyte culture reactions were done only for the two patients with accelerated rejection, and both responses were negative. In a report by Seigler et al on 45 genotypically identical transplant patients, complete family studies, including MLR, had been performed to establish without question the HLA identity of the siblings in question [6]. Of the 45 patients, 23 experienced mild reversible rejection; on average, the rejection episodes began 15 days after transplantation, but all 23 patients subsequently had normal long-term function. Four patients developed acute cellular rejection about 1 month after transplantation and showed satisfactory, but incomplete, recovery. Another five patients with acute cellular rejection returned to normal function. Strikingly, five patients developed acute humoral rejection, confirmed by biopsy; three of the five rejections were irreversible, onset occurring within 5 to 10 days after transplantation. In the same series, six

patients showed evidence of recurrent glomerulonephritis. The overall graft success rate at 2 years was 87%, which is similar to that seen by Cheigh et al [5] and in other published series. In a report on 17 HLA-identical sibling transplant patients, Etheredge et al observed that rejection episodes were more common in five patients with weakly positive MLR responses and that a relatively strong MLR response in one patient was associated with loss of the graft from rejection [7]. Braun and Straffon noted graft loss due to chronic rejection in 4 of 35 HLA-identical sibling transplant patients; the MLR was negative in all 4 patients [8]. In one patient with acute rejection and loss of the graft, there was an HLA-D mismatch although the HLA-A,B,C antigens were identical, an example of a recombinant haplotype. A similar case was reported by Seigler et al in an individual with acute humoral rejection whose MLR was positive with the donor [6]. In our own experience, we are aware of three instances of apparent HLA recombination in which a Class I-identical, MLR (Class II)-incompatible graft was rejected, but there were also two patients in whom rejection did not occur. The frequency of HLA-recombinant transplant patients is still relatively low, but it is clear that rejection does occur when the D locus is mismatched, and the rate seems to be as high as that with HLA-A,B,C,D mismatched patients.

In contrast to some of the cases noted above, there are good serologic data to show HLA identity in the patients under discussion here today. In the first case, the MLR was not performed until after rejection and transplant nephrectomy had occurred. It might be expected that a previously negative MLR may turn positive when HLA-identical rejection has occurred, but this has not been well-documented. The stimulation index of 2.5 and the relative response of 6% has to be considered negative. If the MLR had been performed during the time of acute rejection, we might have expected a false negative response, which has been reported in some patients and demonstrated experimentally in dogs [9]. At the time of rejection, cells that specifically react against donor antigens are not present in the circulation, but are sequestered either in lymphoid tissue or perhaps in the graft. The second patient showed a negative stimulation index of only 1.98, but when calculated as a relative response, it was 29%. We believe the latter method to be more accurate because it is not as influenced by aberrations in the control background cultures. This result suggests that some degree of incompatibility might have been present, but the response is still within the range that we would consider a "match" for the HLA-D locus. The stimulation index is simply the ratio of the counts/min of tritium-thymidine incorporation in the experimental mixture [recipient + donor (mitomycin treated)] compared to the spontaneous counts in recipient cells alone; hence, RDm/R. A better approach is the relative response, which compares the experimental result with a control stimulation that is designated to be maximal. The control stimulus is provided by an unrelated cell donor or a mixture of unrelated cell donors. The background is subtracted from both mixtures; hence, (RDm − RRm)/(RCm − RRm) = relative response.

Mixed lymphocyte culture reaction. The MLR is therefore one approach to assessing incompatibility, which may be significant in unexpected rejection. There are three categories of positive MLR among siblings.

First, there is the clear-cut recombinant between the HLA-B and D loci. In this instance, siblings are serologically identical for HLA-A,B,C, but have strong MLR interactions. As noted above, these transplant patients appear to run an increased risk relative to true HLA identicals.

Second, there may be additional loci for MLR proliferation on chromosome 6, mapping near the HLA-A locus. Recent data on sibling MLR's that indicate HLA recombination are summarized in Table 1 [10]. Eight examples of HLA-B,D recombination had positive MLRs, as expected. Of 14 HLA-A,B recombinant siblings who had matching HLA-B,D antigens and mismatched A antigens, six had positive MLRs. The precise location of this second MLR gene is unknown, and the matter may be complicated by the role of suppressor genes; for example, the MLR may be against the HLA-A antigen itself and is manifest because a suppressor gene for this response has crossed over with the segment containing the B locus. If the locus is outside of HLA-A,B,C,D, siblings could be completely

Table 1. MLR reactivity in HLA-recombinant siblings matched for A and B (recombinants between B and D), or matched for B and D (recombinants between A and B)[a]

	MLR Response	
	Negative	Positive
Recombinant between B & D	0	8
Recombinant between A & B	8	6

[a] Adapted from Ref. 10.

matched for the known antigens, but still have an important incompatibility on chromosome 6, which may or may not be reflected in a weakly positive MLR. Examples of important histocompatibility antigens that are coded for by genes outside of the MHC are now known in experimental animals. A non-H2 skin graft rejection locus is found in the mouse. A similar locus in the rat has recently been localized to the chromosome carrying the MHC, but it is outside of the region bearing the known Class I and Class II antigens [11]. This incompatibility is *not* reflected by significant stimulation in MLR. Chromosomes are inherited intact except when recombination has occurred, and this is more frequent when there is a greater distance between the markers in question. If HLA-B and D are 1 centimorgan apart (1% recombination rate), and a postulated new histocompatibility locus is 10 centimorgans away from HLA, then we might expect a 10% "unexplained" rejection rate.

Third, the presence of antigens from other chromosomes that are capable of stimulating the response may produce a weakly positive MLR. In the mouse, there is such a region called the M locus. Its role in transplantation appears to be relatively minor, and no clear example of such a non MHC locus has been discerned in other species, including man. Nevertheless, this now brings us to consideration of so-called minor transplantation antigens. Preimmunization to multiple minor antigens can result in a state resembling a major histocompatibility barrier. Whether the barrier always consists of a sum total of dozens of such antigens or perhaps a limited number of functionally important antigens is yet to be determined. We must not forget that we continue to rely on immunosuppressive agents in HLA-identical recipients because responses to non-HLA antigenic differences could be significant in every case if untreated.

New approaches in tissue-typing. Clearly, more attention must be paid to detection and characterization of antigenic differences and to states of antigenic presensitization, which are important in patients who are apparently well-matched for the narrow chromosomal region comprising HLA-A,B,C,D. What procedures appear to show promise? The MLR, as reviewed above, could be of some help when the response is weakly positive, but sufficient data are not yet available on the predictive value of MLR alone. The observation that multiply transfused individuals can make a "killer" cell, lymphocyte-mediated cytotoxicity (LMC), response to ^{51}Cr-labeled target cells from HLA-identical siblings is of some importance [12]. When used as a cross-match procedure, this test was the only indication in one patient of a serious anuric cellular rejection, which began about one week after transplantation. HLA-A,B,C,D were matched, and various antibody cross-match tests were negative. This circumstance is not common, and in fact a positive LMC cross match is not consistently predictive of severe rejection in patients mismatched for HLA haplotypes [13]. When a positive MLR has evolved *in vitro,* "killer" cells can be found that can damage donor cells. This assay is called cell-mediated lympholysis (CML) and is generally correlated with the known HLA-A,B,C (Class I) antigenic differences present. Not all killing is predictable by selection of target cells that have the proper antigens, however, and it is therefore likely that the CML test can be used to type for antigens that are important to "killer" cells, some of which are not directed to classical HLA antigens. Another experimental approach to finding "extra" antigens is to restimulate cells that have already responded once in the MLR. In theory, only cells that have the same HLA-D antigens as the stimulators in the first culture will produce an accelerated secondary response. In fact, unexplained "extras" are being observed with this technique, which is called primed lymphocyte typing (PLT).

What serological approaches show promise? We are presently involved in an expanding investigation of a variety of antibody cross-match procedures that offer varying sensitivity and specificity. The main focus is on B cell and macrophage antigens, which are analogous to mouse Ia antigens and closely related if not identical to the Class II MLR-stimulating antigens. A number of antisera have been found that identify serologically the HLA-D locus, and the term HLA-DR is now used for the serologically defined D-related antigens. A number of sera appear to recognize non-D antigens, however, and some of these are *not* mapped to the MHC. Hence, it may be possible to discern antigens that are important in transplantation by testing sera from rejected HLA-identical grafts, not only grafts from kidney recipients, but from bone marrow recipients as well. It is also important to note that macrophages have antigens that are shared with endothelial cells [14]. Some sera from multiparous women have antibodies that react with macrophages and not with B lymphocytes, but they also react with endothelium. The genetics of this system are not yet clear, but it is possible that some HLA-identical rejections may involve responses to this

antigenic system. Presensitization to donor endothelium could occur from macrophages present in blood transfusion, for example.

What might have been done to prevent the difficulties illustrated by these cases? Presently, we still lack effective therapy for rejection when steroids and azathioprine fail. These cases did not suffer "hyperacute" rejections: hence, it is unlikely that cross-match procedures can be definitive in themselves. A concerted effort is needed to define the important antigenic systems that are being missed. These mismatches are at least as important as the standard HLA-haplotype mismatches. Furthermore, means to detect the presence of immunological memory ("presensitization") to these antigens will also be needed.

Questions and Answers

DR. J. J. COHEN: You have estimated that the incidence of early graft rejection is 10%, even when the donor and recipient are HLA-identical siblings and the mixed lymphocyte culture reaction (MLR) is negative. Would skin-grafting be helpful in identifying those at risk of early rejection, or would this technique potentiate the problem by sensitizing the recipient to minor antigens?

DR. C. B. CARPENTER: When I first started as a research fellow, this was exactly how histocompatibility testing was done. The skin grafts were done in the "reverse" direction—recipient to donor—since one didn't want to sensitize the recipient. Reverse skin grafts were done mainly in siblings who appeared to be identical twins, just to be absolutely sure. At that time, there was a meager awareness of major histocompatibility antigens. We knew about identical twins, but there was even some confusion about whether fraternal twins were really different from siblings that were born at different times. It is necessary to be very careful in the determination of end points in skin graft tests because a difference of 2 or 3 days can be important biologically. Reverse skin-grafting might be a nice assay in siblings who are already well-typed for HLA antigens. If recipient and donor are totally HLA identical, skin graft survival will be 28 to 30 days instead of the 10 to 12 days in unrelated individuals. Thus, skin-grafting could be used when donor and recipient are compatible for HLA and in the MLR, for if skin graft rejection occurs at 10 to 12 days, this would indicate that another major incompatibility does exist between donor and recipient. One would hope, however, that the inconvenience and possible hazards of sensitizing a normal sibling could be avoided. I should now like Dr. Marvin R. Garovoy to describe some of our studies in HLA-identical transplant patients who experienced rejection.

DR. M. R. GAROVOY (*Associate Director, Tissue Typing Laboratory, Peter Bent Brigham Hospital*): In the past 1.5 years, we have performed seven transplants in patients who were HLA identical and negative in the MLR. Two recipients had rejection episodes, one mild and one severe, in the first 2 weeks after transplantation. We are employing assays for antibody-dependent lymphocyte-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which can detect the presence of very low levels of antibodies including presensitization with minor antigens. Both of these recipients had positive CDC and ADCC reactions. Therefore, including the patient with positive LMC "killer" cell reactivity mentioned by Dr. Carpenter earlier, we have had three instances of documented presensitization to minor antigens in HLA-identical recipients, and all three patients had early episodes of rejection.

DR. J. J. COHEN: Could rejection have been anticipated in the patients under discussion today if the CDC and ADCC tests had been done before transplantation?

DR. M. R. GAROVOY: Perhaps. The tests were done before transplantation in one of our patients, but we did not appreciate their significance. In the other two patients, the tests were not done before the actual transplantation. In any event, we would not have taken action because we are still collecting data on these tests. Sufficient additional data must be obtained before deciding to implement major changes in the transplant protocol.

DR. C. B. CARPENTER: Another approach to predicting which HLA-identical patients will have rejection may develop from studies of the macrophage endothelium system. If this antigen system becomes well-defined, it may be possible to use the system clinically. Obviously, tissue-typing will become too unwieldy if every minor antigen must be typed. Perhaps there will be only one or two important antigens among the minor antigens that are outside of the HLA region. Finding out which minor antigens are important and typing them could be the final check on HLA-identical recipients. I would much prefer that to skin-grafting.

DR. J. J. COHEN: In view of the apparent physiological function of the Class II antigens, is there any likelihood that we will be able to attack the rejection phenomenon by blocking the expression of these

antigens so that host cells might recognize them differently?

DR. C. B. CARPENTER: Well, some of us think that this is how the phenomenon of enhancement works. In rat models of organ transplantation in which passive transfer of immune antidonor serum blocks rejection, antibodies against Class II antigens seem to be effective, whereas antibodies against Class I antigens are not. We are in the process of working this out in other combinations of inbred rats to see how general a rule this is.

DR. J. T. HARRINGTON: Are experimental transplant models in which donor and recipient are identical for Class II antigens but nonidentical for Class I antigens being used to determine the relative importance of these loci?

DR. C. B. CARPENTER: Yes, we have one such combination of rat under study now, and we find that untreated rejection of kidneys progresses at the usual rate. Susceptibility to rejection modification, however, may turn out to be different.

DR. N. E. MADIAS (NEMCH): Is there any evidence that a difference in sex between the donor and recipient might make a difference in graft survival?

DR. C. B. CARPENTER: The published data on humans, mentioned earlier, do not differentiate the sexes of donor and recipient. In inbred mice, it is interesting that male-to-female grafts show a very slow indolent rejection. The antigen associated with the Y chromosome is thus a minor transplantation antigen. Data in humans about the relative roles of the ABO system [15] and male-to-female or female-to-male renal transplants [8] are still incomplete. In any case, the antigen associated with the Y chromosome is a minor one and is not likely to be the explanation for the 10% incidence of early rejection, as demonstrated by the two patients under discussion, today.

DR. N. E. MADIAS: What are your thoughts regarding transfusions in potential cadaveric transplant recipients? Could this practice promote enhancement?

DR. C. B. CARPENTER: Yes, but it is not clear what is meant by enhancement when it is actively produced. Blood transfusion may be beneficial in some patients and harmful in other patients. I agree with the current position that it is bad to avoid transfusion in a potential cadaveric transplant recipient. The data are quite firm on this.

DR. N. E. MADIAS: Can lymphocytotoxins be isolated from rejected kidney tissue?

DR. M. R. GAROVOY: It is possible, depending on the techniques you use, to isolate antibodies to HLA-A,B,C loci antigens, and HLA-DR locus antigens [16]. When indirect immunofluorescent techniques are performed, these antibodies stain vascular endothelium [17].

DR. J. J. COHEN: Do we know for certain whether the antibodies reach the kidney through the circulation or through local production by cells?

DR. M. R. GAROVOY: Plasma cells actively synthesizing antibodies are present in some grafts; thus, there is the possibility of some local antibody production in the rejecting kidney [18].

DR. J. J. COHEN: Is there a rapid serological test to assay Class II antigens, which could possibly be used in cadaveric transplantation?

DR. C. B. CARPENTER: Yes, this typing is being done on an experimental basis presently. It takes a little longer than the standard HLA typing and specifically identifies the HLA-DR antigens. It is necessary to obtain prospective data from many transplantation cases in order to determine the significance of this kind of matching.

RENAL FELLOW: Is there any work being done to explain how a pregnant woman can maintain a graft in the form of a fetus for nine months without rejection?

DR. C. B. CARPENTER: It is clear that maternal sensitization occurs to fetal HLA antigens during pregnancy. This is, in fact, our main source of HLA-typing reagents. Cellular immunity to HLA has been demonstrated by the sensitive migration inhibitory factor (MIF) assay, and antibodies to HLA, particularly to Class II antigens, can block the MIF response. Since women who are habitual spontaneous aborters lack these blocking antibodies, a form of antibody-induced graft enhancement may be partially responsible for the success of the fetus as an allograft [19]. There are other possibilities as well, and this is a fertile field for investigation.

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